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Personal exposure to environmental tobacco smoke: salivary cotinine, airborne nicotine, and nonsmoker misclassification

ROGER A. JENKINS[a] AND RICHARD W. COUNTS[b]

[a] *Chemical and Analytical Sciences Division, Oak Ridge National Laboratory, PO Box 2008, Oak Ridge, Tennessee 37831-6120*
[b] *Computer Science and Mathematics Division, Oak Ridge National Laboratory, PO Box 2008, Oak Ridge, Tennessee 37831-6418*

A large study was conducted to assess exposure to environmental tobacco smoke (ETS) in a geographically dispersed study population using personal breathing zone air sampling and salivary cotinine levels. Approximately 100 self-reported nonsmoking subjects in each of 16 metropolitan areas were recruited for this investigation. Cumulative distributions of salivary cotinine levels for subjects in smoking and nonsmoking homes and workplaces exhibited a general trend of decreasing salivary cotinine levels with decreasing time spent in smoking environments. Median salivary cotinine levels for the four experimental cells in the study (product of smoking and nonsmoking home and workplaces) were comparable to those reported for a large national study of serum levels of cotinine (Third National Health and Nutrition Examination Survey, NHANES III), when the latter was corrected for expected differences between serum and saliva concentrations. However, the most highly exposed group in this study had a median salivary cotinine concentration approximately a factor of 2 greater than that of the comparable group in the NHANES III study. Misclassification rates, both simple (for self-reported nonsmokers) and complex (self-reported lifetime never smokers), were near the median of those reported for other studies. Estimated misclassification rates for self-reported lifetime never-smoking females are sufficiently high (2.95% using a discrimination level of 106 ng/ml) that, if used in the Environmental Protection Agency (EPA) risk assessment related to ETS and lung cancer, would place the lower 90% confidence interval (CI) for relative risk at nearly 1.00, i.e., no statistically significant increased risk. For the 263 most highly exposed subjects in the study whose self-reported nonsmoking status was accurate, the correlation between airborne exposure to nicotine and average salivary cotinine is so small, on an individual basis, that it makes the relationship useless for estimating exposure on a quantitative basis. When subjects are grouped according to likely categories of nicotine exposure, correlation between group median airborne nicotine exposure and salivary cotinine level increases dramatically. The comparison improves for the most highly exposed subjects, suggesting that such quantitative comparisons are useful for only those subjects who are exposed to the higher levels of ETS. However, airborne nicotine exposure for most of the subjects does not account for estimated systemic levels of nicotine, based on salivary cotinine levels.

Keywords: *cotinine, environmental tobacco smoke, nicotine, personal exposure.*

Introduction

Cotinine is one of the primary metabolites of nicotine, and its concentration in body fluids (serum, saliva, and urine) is believed to be a reliable indicator of the extent of passive exposure to environmental tobacco smoke (ETS). While many studies have examined its relationship to some indicators of ETS exposure (time spent around smokers, number of cigarettes observed, number of smokers in one's residence, etc.) (e.g., Jenkins et al., 1992; Delfino et al., 1993; Emmons et al., 1994; Rebagliato et al., 1995; Thompson et al., 1995; Tunstall-Pedoe et al., 1995; Emmons et al., 1996), few studies have provided an assessment in nonsmokers of cotinine levels vs. airborne nicotine (e.g., Proctor et al., 1991; Marbury et al., 1993; Ogden et al., 1993; Bergman et al., 1996; Phillips et al., 1996, 1997, 1998a, 1998b, 1998c). Nicotine is the primary precursor to cotinine (Curvall et al., 1990; Byrd et al., 1992). One interesting assessment used area monitoring levels of nicotine in workplaces to model personal exposure, and compared the results with salivary cotinine from another study population to model risk from ETS exposure in the workplace (Repace et al., 1998). Relatively few studies (Proctor et al., 1991; Phillips et al., 1997, 1998a, 1998b, 1998c) have compared results from personal breathing zone sampling of ETS nicotine, and salivary cotinine (the matrix that is easiest to collect from subjects), and none has reported on data on a geographically dispersed population within a given society. The purpose of the work reported here is to describe findings of a large, 16-city study within the United States, with regard to salivary cotinine levels and

1. Abbreviations: CI, confidence interval; EPA, Environmental Protection Agency; ETS, environmental tobacco smoke; h, hour; LOD, limit of detection; NHANES III, Third National Health and Nutrition Examination Survey; ORNL, Oak Ridge National Laboratory; QA, quality assurance; QC, quality control; RIA, radioimmunoassay; RJRT, R.J. Reynolds Tobacco Co., Inc.; RSD, relative standard deviation; TWA, time-weighted average.

2. Address all correspondence to: Roger A. Jenkins, Mail Stop 6120, Building 4500S, Oak Ridge National Laboratory, Bethel Valley Rd., PO Box 2008, Oak Ridge, TN 37831-6120. Tel.: (423)576-8594. Fax: (423)576-7956. E-mail: jenkinsra@ornl.gov

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direct determination of ETS nicotine (predominantly vapor phase, *ca.* 95+%, Eudy et al., 1986; Benner et al., 1989; Eatough et al., 1989) exposure. Particular emphasis is given to determination of nonsmoker misclassification rates and a comparison of inhaled vs. systemic nicotine.

Methods

The experimental design, subject recruiting and resulting demographics, the experimental methods, and the participation of the Research and Development Department of R.J. Reynolds Tobacco Co., Inc., (RJRT/RD) and Bellomy Research have been described in detail elsewhere (Jenkins et al., 1996). Briefly, approximately 100 nonsmokers (18 or older) were recruited, predominantly through the use of random telephone dialing or reverse databases, from each of 16 cities in the 48 contiguous United States. To be eligible for the study, subjects had to be nonsmokers for at least 6 months prior to the study and not be using a nicotine patch or gum. They had to work outside the home on a 'regular' shift (*ca.* 8 a.m. to 5 p.m.) at least 35 h/week.

Subjects arrived at a test coordination center in the early evening of Day 1, and were re-screened (verifying the responses on the telephone-administered questionnaire) prior to study inclusion. Subjects provided a saliva sample between 8:15 and 9:00 p.m. on the evening of Day 1. Subjects initiated their breathing zone personal air sampling, using a workplace sampling pump, at the beginning of their work day on Day 2, usually between 7 and 9 a.m. At the end of their workday, subjects turned off the workplace sampling pump, and turned on the away-from-work sampling system. (The latter was identical to the former, except for a larger battery pack to provide power for a minimum of 18 h.) The away-from-work sampling system was to remain on the subject, except for bathing and sleep periods, when it was near the subject, until the subject arrived at work on the morning of Day 3. At that time, the away-from-work sampling system was turned off. Subjects returned to the test coordination site between 4 and 6 p.m. on the evening of Day 3, and provided a second saliva sample. Thus, the Start and End saliva samples bounded a period of 45 h, while the actual time of personal exposure measurement occupied a 24-h block (~9 h for workplace sampler and ~15 h for the away-from-work sampler) in the approximate center of the 45-h window. Given the half-life of cotinine in nonsmokers (Benowitz, 1996), the time within the 45-h window when personal monitoring was not conducted was not expected to contribute to important changes in the concentrations of salivary cotinine, unless the subject received an exposure to ETS during the Day 3 workday which was radically different from that received on the Day 2 workday.

The personal air sampling equipment has been described in detail elsewhere (Ogden et al., 1996). It consisted of a sound-insulated sampling pump (approximately 8.5 in.×6.5 in.×4.25 in., weighing *ca.* 3.5 lb) attached to a clip-on sampling head. The pump was mounted in a plastic case, affixed to which were shoulder straps, and participants were requested to wear the pump with the strap over the right shoulder and the pump resting on the left hip. The sampling head contained both particle and vapor phase ETS marker collection devices and was designed to be worn in a person's 'breathing zone,' i.e., clipped onto a shirt collar, lapel, or pump shoulder strap within 25 cm of the mouth. Vapor phase nicotine was collected on XAD-4 resin cartridges (SKC, Eighty-Four, PA) at a flow rate of approximately 0.5–0.7 l/min.

Saliva samples were collected using Salivettes (Sarstedt, Newton, NC), which were comprised of a sterile cotton cylindrical swab contained inside a clear plastic centrifuge tube. Samples were collected by having the subjects open the plastic tube and drop the cotton into their mouths without touching the swab. The cotton was chewed vigorously for 60 s, and expelled back into the tube without hand contact. Samples (saliva and breathing zone nicotine) were stored frozen upon receiving them from the participants, shipped to the analytical laboratories on dry ice, and stored in freezers until analyzed. Levels of salivary cotinine were determined using radioimmunoassay (RIA) (Davis and Stiles, 1993), using the procedure developed by Van Vunakis et al. (1987, 1993) within the laboratories of RJRT/RD, operating under an agreement of cooperation with the sponsor of this study (see Acknowledgement). Each sample was analyzed in duplicate using a 13-point calibration fitted to a cubic spline curve in city-by-city batches. Relative standard deviations (RSD) of duplicate sample analysis results for a given batch ranged from 11.5% to 24.7%. The mean RSD for all sample duplicates (with 21 of 2751 samples deemed to be outliers and excluded) was 18.1%. Quality assurance/quality control (QA/QC) measures used to confirm the accuracy of the results reported for cotinine included the analysis of ten field blank samples per batch (five 'start' and five 'end'), analysis of six buffer samples spiked with cotinine (two each at *ca.* 1.5, 15, and 30 ng/ml), and analysis of four actual nonsmoker, non-ETS-exposed saliva samples spiked with cotinine (two each at *ca.* 2 and 13 ng/ml).

Vapor phase nicotine samples were also determined by methods previously described (Ogden, 1991) by RJRT/RD. A variety of QA/QC procedures were instituted to independently insure the reliability of the data (Jenkins et al., 1996). These included shipping to RJRT/RD for blind analysis saliva samples from practicing smokers and known nonsmokers for a qualitative assessment of the accuracy of the cotinine determination, as well as solutions, solution-spiked XAD-4 traps, and ETS-loaded traps for the nicotine determinations. Performance of the laboratory on all these blind samples/solutions was very good.

Results and discussion

Distribution of Salivary Cotinine Concentrations

Salivary cotinine levels encountered in the study were widely distributed. In Figure 1 are summarized cumulative distributions for all subjects for which cotinine levels were available (N=1486) by self-reported cell classification. For this study, Cell 1 was populated by subjects living and working in smoking environments; Cell 2, by subjects living in smoking home, but working in nonsmoking workplaces. Cell 3 was populated by subjects living in nonsmoking homes, and working in smoking workplaces; while Cell 4 was populated by subjects both living and working in nonsmoking environments. Average levels (the mean of the start and end analyses) ranged from below the mean limit of detection (LOD) of 0.550±0.263 ng/ml to nearly 1050 ng/ml. There was a clear trend towards decreasing cotinine concentrations with decreasing time spent in smoking environments, as the median level of Cell 1 subjects is greater by approximately a factor of 2 than that of subjects in Cell 2, and so on. This factor of 2 difference between adjacent cells is maintained across the central 50% (i.e., 30% to 80%) of the distributions. However, more than 63% (938 of 1486) of the subjects had concentrations below the mean LOD. Approximately 25% of the subjects (365 of 1486) had average salivary cotinine concentrations above the upper 95% confidence bound above the mean LOD (equal to 1.01 ng/ml). If a level of 15 ng/ml (Etzel, 1990) is employed to discriminate between nonsmokers and occasional smokers, 31% of the subjects (i.e., 466 subjects between the mean LOD and the discrimination level) were nonsmokers and had salivary cotinine levels which would

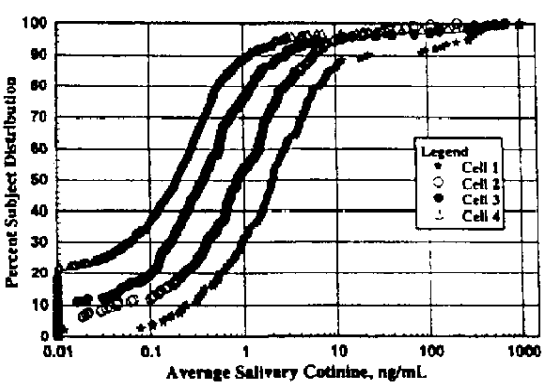

Figure 1. Cumulative distribution of all study subjects as a function of average of start and ending salivary cotinine level, by cell. Note log scale on the horizontal axis. Cell 1: smoking home/smoking workplace. Cell 2: smoking home/nonsmoking workplace. Cell 3: nonsmoking home/smoking workplace. Cell 4: nonsmoking home/nonsmoking workplace.

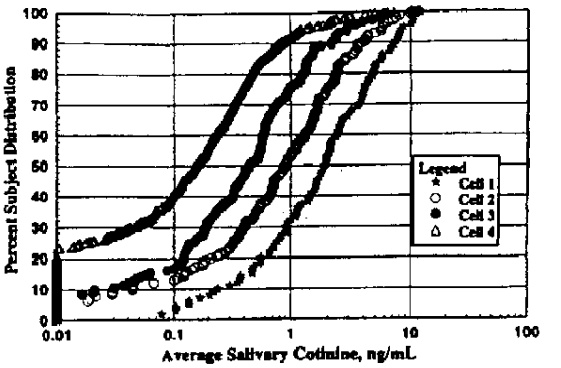

Figure 2. Cumulative distribution of all study subjects (confirmed to be nonsmokers, based on a salivary cotinine level < 15 ng/ml) as a function of average of start and ending salivary cotinine levels, by cell. All subject cell placements confirmed by diary reports of smoking or lack thereof in the particularly venue. Note log scale on the horizontal axis. Cell 1: smoking home/smoking workplace. Cell 2: smoking home/nonsmoking workplace. Cell 3: nonsmoking home/smoking workplace. Cell 4: nonsmoking home/nonsmoking workplace.

be attributable to indirect exposure to nicotine. In Figure 2 are reported the cumulative distributions of salivary cotinine for subjects with salivary cotinine less than 15 ng/ml, and whose self-reported cell assignment was congruent with their observations of smoking in their home or workplace. The requirement of congruence does not alter the general shape of or conclusions drawn from the distributions.

With the exception of subjects reporting exposure in *both* the home and workplace, the median levels of salivary cotinine, grouped according to cells, are quite similar to the geometric mean levels reported by Pirkle et al. (1996) for workers of age 17 or older in a national study (the Third National Health and Nutrition Examination Survey, NHANES III), where the latter were converted from serum cotinine levels to associated salivary cotinine levels, according to Benowitz (1996). In Table 1 are reported median levels of subjects claiming to be nontobacco users (all subjects), those subjects whose saliva cotinine levels were below the smoker/nonsmoker discrimination level of 15 ng/ml, and a subset of the latter group whose recorded observations of ETS exposure on diaries were congruent with their previous self-reported cell assignments. (Note that median levels were used, rather than geometric means, because a large number of the subjects had salivary cotinine levels which were undetectable. Geometric means require that all considered values are nonzero, which was not the case for our study.) Both studies show consistently decreasing cotinine levels with decreasing time spent in smoking environments. The extent to which cross-reactivity (the affinity of the anti-cotinine antibodies for the *trans*-3-OH-cotinine [t3OHcot] or other nicotine metabolites in the RIA) may play a role in this difference is difficult to

Table 1. Comparison of salivary cotinine median and geometric mean levels for working subjects (NHANES III versus this study).

ETS exposure venue	Cell designation	NHANES III (Pirkle et al., 1996) [*N*=2672]	This study [*N*=1486]		
		Geometric mean levels, estimated from serum levels[a]	Median level, all subjects	Median level, subjects with salivary cotinine below misclassification discrimination level[b]	Median level, subjects with salivary cotinine below misclassification discrimination level[b] and ETS exposure observations consistent with self-reported cell assignment
Exposed at both home and work	1	1.16	2.06	1.78	1.97
Exposed at home only	2	0.81	0.86	0.82	0.89
Exposed at work only	3	0.40	0.37	0.34	0.44
Unexposed at either home or work	4	0.16	0.19	0.18	0.15

[a]Estimated using the expression saliva cotinine = 1.25 × serum cotinine (Benowitz, 1996).
[b]Misclassification discrimination level: saliva cotinine ≥15 ng/ml.

determine. First, while the amount of t3OHcot, relative to that of cotinine in *urine* may be comparable or greater (Zuccaro et al., 1997), the relative proportion in saliva appears to be much lower (Phillips, 1998). Secondly, if cross-reactivity was accounting for the difference between results reported by Pirkle et al. (1996) and those reported here for the Cell 1 subjects, the differences would be expected to exist for subjects in Cells 2 and 3. Such is clearly not the case. The reasons for the differences between the NHANES data and that from this study for the most highly exposed subject group (based on self-reported time spent in smoking environments) are not clear, but may be due to differences in subject population definition. (In our study, all subjects were 18 or older and had to work outside the home a minimum of 35 h/week on a regular shift. Reduced time in smoking workplaces may contribute to reduced overall salivary cotinine levels.)

Misclassification

Subjects whose salivary cotinine levels were indicative of their direct exposure to tobacco products (smoking, chewing, of snuff dipping) or smoking cessation aids (nicotine patch or gum) were excluded from additional comparisons with airborne ETS markers. Establishing the fraction of subjects which were misclassified as nontobacco users is dependent upon the discrimination level of salivary cotinine employed (Etzel, 1990). In this study, the simple misclassification rates (number of subjects above a given discrimination level divided by the total number of subjects for which salivary cotinine levels were available) ranged from 2.2% to 6.6%, depending on gender and discrimination level (Table 2). These levels are comparable to those reported in a large summary of misclassification rates (Lee and Forey, 1995). The data also suggest that males exhibited a somewhat greater misclassification rate than females, which has also been reported previously (Lee and Forey, 1995). Riboli et al. (1995) reported a simple misclassification rate of 1.5% for *ca.* 1400 women based on urinary cotinine. Fontham et al. (1994) reported a simple misclassification rate of 1.9% in a large epidemiologic study of females, based on urinary levels of cotinine. This is similar to that of females in this study at the highest discrimination

Table 2. Estimation of simple misclassification rate of study subjects[a] from salivary cotinine level.

Salivary cotinine level (ng/ml)	Females above discrimination level	Female simple misclassification rate (%)	Males above discrimination level	Male simple misclassification rate (%)	Overall misclassification rate (%)
Mean≥100	22	2.20	17	3.51	2.62
Mean≥35	30	2.99	19	3.93	3.30
Mean≥30	31	3.09	22	4.55	3.57
Mean≥15	39	3.89	27	5.58	4.44
Mean≥10	45	4.49	32	6.61	5.18

[a]Number of study participants for whom cotinine and demographic data were available: 1486.

level in Table 2 (salivary cotinine >100 ng/ml). Wagenknecht et al. (1992) have reported a simple misclassification rate (using a serum cotinine level of 14 ng/ml—equivalent to a saliva level of 17.5 ng/ml [see below]) of 4.2% for subjects claiming to be nonsmokers, and a rate of 2.6% for subjects claiming to be never-smokers. In a study of *ca.* 200 subjects in Turin, Italy, Phillips et al. (1997) have reported nonsmoker misclassification rates ranging from 1.6% to 6.1%, depending on the discrimination criteria.

In epidemiologically based risk assessments, the rate at which current active smokers misreport themselves to be lifetime never-smokers is an important confounder for which adjustment is required if an accurate estimate of the relative risk is to be made. There are many approaches to estimation of this misclassification rate. In its risk assessment related to ETS exposure and lung cancer (US Environmental Protection Agency, 1992), the US Environmental Protection Agency (EPA) defined a regular smoker misclassification rate as the ratio of misreporters to the number of smokers who are 'regular' smokers. In this case, the fraction of current smokers who are 'regular' smokers is defined as those whose salivary cotinine level is greater than 30% of the mean level found in all self-reported smokers. Based on a study reported by Ogden et al. (1997), the discrimination level would be 106 ng/ml to define a 'regular' smoker. This level is not inconsistent with the >100 ng/ml discrimination level recommended by Etzel (1990) of the Centers for Disease Control.

Estimating this misclassification rate from the data in the study reported here requires that, since only nonsmokers were recruited, the number of smokers who 'would have been encountered' must be estimated from the following equation:

$$\frac{N}{1-S}SR,$$

where N=number of subjects in the study; S=fraction of individuals in the general population who smoke at all; R=fraction of smokers who are 'regular' smokers.

Data obtained from a survey of smoking rates in 1993 (when much of the field work for this study was performed) indicated that 25.0% (95% confidence interval (CI): ±0.7%) of adults in the United States were current smokers; 20.4% (95% CI: ±0.7%) were daily smokers, and 4.6% (95% CI: ±0.3%) were occasional (not every day) smokers. Smoking prevalence was higher among males (27.7%) than females (22.5%) (US Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, 1994).

In our study, salivary cotinine data and demographic data were available for 1486 subjects. The number of individuals whose salivary cotinine levels indicated that they were regular smokers was divided by an estimate (based on the above percentages) of the number of smokers who *would have been* encountered to produce a misclassification rate. Obviously, the rate is highly dependent on the level of salivary cotinine which is chosen to indicate status as a smoker. Etzel (1990) has reviewed a variety of studies which have addressed discrimination levels for assignment of individuals to smoking categories based on cotinine levels in physiological fluids. Those subjects with salivary cotinine between 10 and 100 ng/ml were classified as infrequent smokers, or regular smokers with low nicotine intake. Those individuals with salivary cotinine >100 ng/ml were classified as regular smokers. Those with salivary cotinine levels <10 ng/ml were classified as probably having no nicotine use, and thus would have received all nicotine through ETS exposure or dietary sources. In Table 3, misclassification rates of subjects claiming to be lifetime never-smokers have been presented for five different discrimination levels. According to the EPA, the definition of regular smokers includes only those subjects whose physiological cotinine levels exceed 30% of the mean level found in all self-reported smokers. The discrimination level for subjects who are considered to be 'occasional smokers' is 10% of the mean level. In a national misclassification study, Ogden et al. (1997) found the mean level for salivary cotinine to be *ca.* 350 ng/ml for all subjects self-reporting to be smokers. Thus, discrimination levels of 106 ng/ml and 35 ng/ml were used. Estimated misclassification rates pre-

Table 3. Estimation of misclassification rate of subjects claiming to be lifetime never-smokers from salivary cotinine level.

Salivary cotinine level (ng/ml)	Females claiming to be never-smokers	Estimated female misclassification rate (%)	Males claiming to be never-smokers	Estimated male misclassification rate (%)	Overall estimated misclassification rate (%)
Mean≥106	7	2.95	6	3.97	3.22
Mean≥35	9	3.79	7	4.63	3.96
Mean≥15	11	4.63	9	5.95	4.95
Mean≥10	13	5.48	11	7.27	5.94
Start or finish level≥10	17	7.16	13	8.59	7.42

Number of study participants for whom cotinine and demographic data were available: 1486.
Assumes fraction of smokers in general population equals 25.0%, and that 81.6% of all smokers are 'regular smokers.' Male smoking rate: 27.7%; female smoking rate: 22.5%. Number of males: 484; number of females: 1002.

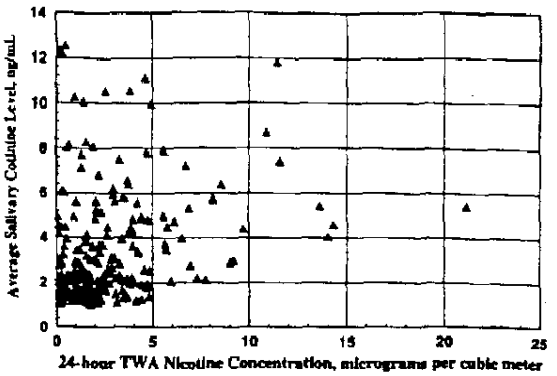

Figure 3. Comparison of individual average salivary cotinine levels with 24-h TWA nicotine concentrations for subjects whose cotinine and 24-h TWA nicotine levels were greater than the 95% confidence level above the mean limit of detection (see text).

sented in Table 3 ranged from 3% to 7% for subjects whose mean of start and end levels was ≥10 ng/ml. The misclassification rate of males tended to be higher at any given discrimination level than that of females, usually by 1.0–1.5%. This phenomenon has been reported previously (Lee and Forey, 1995).

At the highest discrimination level (106 ng/ml), the misclassification rate for lifetime never-smoking females (2.95%) is substantially greater than that estimated by the EPA (1.09%) in its risk assessment of ETS exposure and respiratory disease (US Environmental Protection Agency, 1992). At the misclassification rate determined for this study, the lower 90% confidence bound on the relative risk estimated by EPA from a meta-analysis of epidemiological studies approaches 1.00, suggesting no statistically significant increase in relative risk of lung cancer due to exposure to ETS. (It should be noted here, that there may be important demographic differences between the subjects in this study and those in studies relied upon by the EPA in its risk assessment which could impact misclassification rates. For example, in this study, subjects were required to work outside the home, whereas in studies relied upon by the EPA, a relatively high proportion of subjects were housewives.) The overall combined misclassification rate estimate for males and females at the highest discrimination level in Table 3, calculated using the EPA approach, is 3.22%. This is similar to, but somewhat higher than that calculated by Ogden et al. (1997) (2.81%) using a population weighted method. Differences between estimates from our study and that reported by Ogden et al. (the largest US study of misclassification to date) may be due to differences in the representativeness of the subject populations in the two studies to the US population as a whole.

Comparison of Salivary Cotinine Levels and Nicotine Exposure

In general, the levels of cotinine found in the saliva of the subjects were very low. Since the levels of nicotine exposure, as indicated by 24-h time-weighted average (TWA) concentrations, were also low, the number of subjects whose salivary cotinine and 24-h TWA level of nicotine exposure which were sufficiently high for confident comparison was not large. In Figure 3 are presented the data for 263 subjects whose 24-h TWA nicotine levels and salivary cotinine concentrations were greater than the upper 95% confidence bound above the mean LOD for each of the two constituents. Visually, there is virtually no correlation between these two indicators of ETS exposure on an individual basis. In Table 4 are presented the correlation coefficients for each cotinine level determined, when regressed on the 24-h TWA nicotine levels, for the subject pool described for Figure 3. With the exception of the comparison between the Start and End Difference, all the correlation coefficients are statistically different from zero at the 95% confidence level. However, all the values are low, and none of the R^2 values exceeded 0.11. For the regression of average salivary cotinine on nicotine, the R^2 value was 0.105.

To assess the extent to which uncertainties in the value of cotinine could be responsible for the apparent lack of

Table 4. Correlation coefficients: salivary cotinine vs. 24-h TWA nicotine subjects whose levels are both greater than 95% CI above mean LOD for constituent (N=263).

Regression	Correlation coefficient (R)	p-value[a]	95% CI for R[b]	Coefficient of determination (R^2)
Starting cotinine vs. 24-h TWA nicotine	0.200	p=0.001	(0.081, 0.313)	0.040
Ending cotinine vs. 24-h TWA nicotine	0.327	p<0.001	(0.215, 0.431)	0.107
Average cotinine (mean of Start and End) vs. 24-h TWA nicotine	0.324	p<0.001	(0.211, 0.428)	0.105
ΔCotinine (End–Start) vs. 24-h TWA nicotine	0.114	p=0.065	(−0.007, 0.232)	0.013

[a]Significance level for two-tailed test of hypothesis: H_0: R=0 vs. alternative hypothesis: H_A: $R \neq 0$.
[b]95% CI for the correlation coefficient R.

correlation between 24-h nicotine levels and salivary cotinine, an analysis of random variation of reported cotinine levels was performed. Assuming an exact linear relationship between nicotine and cotinine, the average ratio of cotinine to nicotine, R_{AV} was computed to be 5.20. Next, estimated cotinine levels (COT_c) were calculated from measured 24-h TWA nicotine values from each subject (NIC_{24}), according to $COT_c = R_{AV} \times NIC_{24}$. The mean calculated cotinine value (COT_{cAV}) was determined to be 12.084 ng/ml. A least-squares fit of COT_c vs. NIC_{24} ($COT_c = a + b \times NIC_{24}$) yields $a = -2.1 \times 10^{-14}$ (i.e., essentially 0); b=5.204158 (i.e., R_{AV}); R^2=1.000.

Next, a random perturbation was added to the COT_c values. For each COT_c value, a random perturbation, ξ, was generated from a normal distribution having a mean=0 and a standard deviation of σ_ξ. The standard deviation, σ_ξ, was defined as a percentage of the average corrected cotinine ($\sigma_\xi = \sigma_{\%relative} \times COT_{cAV}$). A starting value of $\sigma_{\%relative}$=10% was used and a least-squares fit was made to the perturbed data. The fit using an RSD of 10% resulted in an R^2=0.993. This was repeated for increasingly greater values of $\sigma_{\%relative}$ until R^2 decreased to approximately zero. For example, at $\sigma_{\%relative}$=100%, $R^2 \approx 0.6$; at $\sigma_{\%relative}$=200%, $R^2 \approx 0.27$; at $\sigma_{\%relative}$=350%, $R^2 \approx 0.1$. In other words, $\sigma_{\%relative}$ would have to be approximately 350% for the R^2 to drop to the level observed in this study. Since the observed imprecision (RSD) in the cotinine analyses was <25%, it can be concluded that the lack of correlation between the salivary cotinine and nicotine exposure is not due to random variation in cotinine analyses.

Another explanation for the lack of correlation between salivary cotinine and nicotine exposure may be that any long delay between the last air nicotine collection and the final saliva sample acquisition may contribute to changes in salivary cotinine level. This could, in turn, contribute to a lack of correlation between nicotine exposure and salivary cotinine. If such were an important factor, subjects with exposure to ETS in both the home and workplace (Cell 1) would be expected to have an improved correlation between 24-h TWA nicotine and *ending* levels of cotinine. In fact, for those Cell 1 subjects of the 263 for whom ending salivary cotinine levels were available (N=75), R^2=0.122. Segregating these subjects into exposure cells produced no important increases in correlation between nicotine exposure and average (of start and ending samples) cotinine levels. Coefficients of determination (R^2) were 0.119, 0.076, 0.033, and 0.126 for subjects in Cells 1–4, respectively.

Presumably, the impact of individual differences in metabolism is more important than actual nicotine exposure in the determination of salivary cotinine for subjects on an individual basis. Interestingly, Phillips et al. (1997) have reported much better correlation between 24-h TWA nicotine and post-test salivary cotinine for a pool of 69 subjects in Turin, Italy (R^2=0.527). However, the reported 24-h TWA levels for subject pools equivalent to Cells 2 – 4 in this study were two to eight times higher in the Turin study, and may account in part for the improved correlation.

The proportionality of salivary cotinine and nicotine exposure increases considerably if the subjects are clustered in groups in order of increasing nicotine exposure, and the *median* salivary cotinine levels are compared with the *median* 24-h TWA nicotine levels for the group. For groups of 25 (with the exception of the group with the highest median nicotine, for which N=13), the correlation between the median cotinine and nicotine levels in each group increased to R^2=0.915. These results are presented graphically in Figure 4 .

In Tables 5 and 6 are presented data regarding the relationship between the median average salivary cotinine level and the median 24-h TWA level of nicotine to which the subjects were exposed as a function of cell designation. (Note that in Table 5, only the responses to the screening questionnaire were used to determine cell assignments. In the second comparison (Table 6), the cell assignments were based both on the response to the screening questionnaire and on diary observation confirmation of the presence/absence of smoking products in the venue in question.) For relatively large groups such as this, the correlation is very high (R^2=0.992 for the data sets in Tables 5 and 6). Note also that for the data set in which only screening questionnaire responses are used for cell assignments (Table 5), the median 24-h TWA nicotine levels and salivary cotinine levels are lower than those for which consistency exists between cell assignment and observation of tobacco product smoking around the subjects (Table 6). This indicates that the actual at-work, or away-from-work observations, of

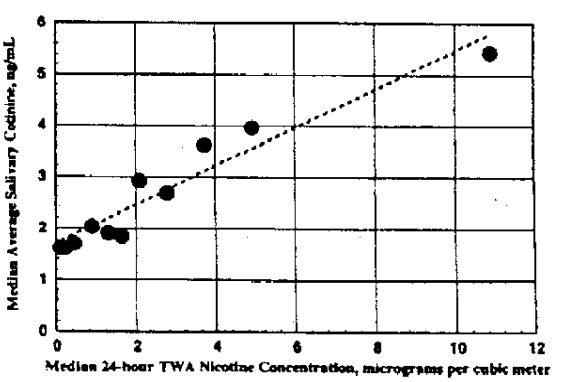

Figure 4. Median salivary cotinine levels as a function of median 24-h TWA nicotine levels for groups of 25 subjects, grouped according to descending nicotine level. Note that the subject cotinine levels ranged from 1.01 to 15 ng/ml. The dashed line is a least-squares linear fit of the reported data.

Table 5. Comparison of salivary cotinine levels and 24-h TWA nicotine levels among cells classified by screening questionnaire only.

Cell	Away-from-work environment	Work environment	Number of participants	Median cotinine (ng/ml)[a]	Median 24-h TWA nicotine level ($\mu g/m^3$)
1	smoking	smoking	133	1.78	1.55
2	smoking	nonsmoking	213	0.807	0.49
3	nonsmoking	smoking	266	0.347	0.11
4	nonsmoking	nonsmoking	790	0.182	0.03

[a]Cotinine results used in this calculation are the mean of Start and End determinations.

some subjects are not congruent with their on-going impression of their workplace or home smoking status.

Material Balance

A number of investigators have either assumed or concluded that body fluid nicotine levels can be a quantitative indicator of ETS exposure (Cummings et al., 1990; Benowitz, 1996; Jaakkola and Jaakkola, 1997). However, few studies have been published where both personal nicotine exposure and body fluid levels of cotinine have been determined directly. Proctor et al. (1991) observed detectable salivary cotinine levels in all, but one of 52 British nonsmoking women, and detectable nicotine exposures in 22 of the 52 subjects. Marbury et al. (1993) compared urinary cotinine levels with integrated activity room levels of ETS nicotine for 48 pre-school age children. Ogden et al. (1993) reported 5-day duration personal nicotine levels and salivary cotinine levels for 96 nonsmoking married women in Columbus, OH. Phillips et al. (1996, 1997) have reported on the nicotine exposure and salivary cotinine levels of 190 nonsmokers in Stockholm, Sweden, and 188 nonsmokers in Turin, Italy. A recent series of manuscripts by these same authors has reported nicotine exposures and salivary cotinine levels for subjects in the cities of Lisbon, Bremen, and Paris (Phillips et al., 1998a, 1998b, 1998c).

It has been estimated that the conversion factor which relates a blood level of cotinine to a daily intake of nicotine is 80 $\mu g/24$ h/ng/ml (Benowitz, 1996). While this conversion factor has been determined for smokers, it has been believed to be applicable to nonsmokers (Benowitz, 1996). Thus, a serum cotinine level of 3 ng/ml would correspond to a daily intake of 240 μg nicotine. Factors which relate salivary levels of cotinine to those of serum range from 1.1 to 1.4 (Benowitz, 1996), or an average of 1.25. Using these factors, a salivary cotinine level of 3.75 ng/ml would be calculated to represent an intake of 240 μg of nicotine per day. A material balance between inhaled nicotine and systemic nicotine is not supported by the direct determinations reported in this study, however. In Table 7 are reported comparisons between the apparent systemic dose (comparable to "total nicotine exposure" described by Chappell and Gratt (1996)) of nicotine, and potential inhaled quantity of nicotine, presumably all from ETS exposure. In order to make confident comparisons, only those subjects (N=263) in the study whose average salivary cotinine level and 24-h TWA nicotine levels were greater than the upper 95% confidence level about the mean LOD (1.01 ng/ml and 0.053 $\mu g/m^3$, respectively) were included in this data analysis. The data in Table 7 indicate that, across the entire distribution, the amount of nicotine inhaled, based on a reasonable subject activity scenario, is only a small fraction of the systemic dose of nicotine estimated from salivary cotinine levels. When the inhaled nicotine dose is corrected for estimated absorption efficiency (*ca.* 71%, Benowitz, 1996), the median ratio of systemic dose to inhaled (absorbed) dose is nearly a factor of 10. In other words, if the proposed model as described in Benowitz (1996) is accurate, only a small fraction (*ca.* 10%) of the estimated systemic dose can be ascribed to inhaled ETS nicotine.

The conclusion, that the inhaled dose is only a fraction of the systemic dose, is not particularly sensitive to changes in human activity patterns or reasonable human breathing

Table 6. Comparison of salivary cotinine levels and 24-h TWA nicotine levels among cells classified by screening questionnaire and diary observations of tobacco products.

Cell	Away-from-work environment	Work environment	Number of participants	Median cotinine (ng/ml)[a]	Median 24-h TWA nicotine level ($\mu g/m^3$)
1	smoking	smoking	100	1.94	2.00
2	smoking	nonsmoking	138	0.879	0.73
3	nonsmoking	smoking	144	0.446	0.16
4	nonsmoking	nonsmoking	545	0.163	0.03

[a]Cotinine results used in this calculation are the mean of Start and End determinations.

Table 7. Comparison of calculated nicotine doses and ratio of dose estimated from salivary cotinine levels to that estimated from personal exposure determinations.

	Calculated potential inhaled quantity of nicotine from personal monitoring[a] (μg/day)	Estimated systemic nicotine dose[b] (from Benowitz, 1996) (μg/day)	Ratio of estimated systemic dose and calculated potential inhaled quantity, corrected for absorption factor[c]
Median	22.8	141	9.9
Mean	36.4	201	31.6
20th Percentile	4.1	91	4.2
80th Percentile	58.8	293	39.9
95th Percentile	114	516	134

[a]Calculated using breathing rate recommendations from the EPA Draft Exposure Factors Handbook (1996) and individual sleep, work, and away-from-work duration reported by subjects. Estimated sedentary time at home: 2 h; estimated sedentary time at work: 4 h.
[b]$N=C_s[(80\ \mu g/day/ng/ml)/1.25]$, where N is the estimated systemic nicotine dose (μg/day), C_s is the salivary cotinine level. See text for explanation of conversion factors.
[c]Absorbed nicotine taken to be 71% of inhaled nicotine.

rates. Thus, this finding suggests that a number of assumptions of, or inputs to, this type of model may not be accurate. The most plausible explanations for the discrepancies include (a) over-reporting of actual salivary cotinine concentration, due to cross-reactivity in the RIA analyses performed; (b) large differences in metabolism between smokers and nonsmokers (the former for which most of the metabolic pathways and rates have been determined); or (c) underestimating the relative importance of dietary or other nonairborne sources of nicotine.

If cross-reactivity was causing a serious overestimation of cotinine levels in saliva, it seems unlikely that the data reported for this study would be in such good agreement (within a factor of 2) with that reported for the NHANES III survey (Pirkle et al., 1996). In addition, the salivary cotinine levels of more highly ETS exposed subjects are within the range observed by other investigators (Lee and Forey, 1995). Thus, overestimation is probably not a substantial contributor to the apparent discrepancy between inhaled nicotine and systemic nicotine.

For subjects with quantifiable salivary cotinine and airborne nicotine exposures (Table 7), accounting via dietary intake for the remaining 90% of systemic levels of nicotine which cannot be ascribed to airborne nicotine seems implausible. If dietary sources of nicotine (Davis et al., 1991) were playing an important role in systemic levels of nicotine (and by inference, salivary cotinine) for these subjects, it would also be expected that, for a substantial fraction of Cell 4 subjects reporting no ETS exposure, salivary cotinine levels would be much greater than 1.0 ng/ml. (Substantial dietary pattern differences between the Table 7 subjects and those in Cell 4 could negate this conclusion. However, an analysis of dietary patterns [self-reported consumption of green or regular tea, vegetable juices, etc.] indicates virtually no differences between these two groups.] Yet an examination of the cotinine distribution for Cell 4 subjects portrayed in Figure 2 indicates that less than 8% of subjects have an average salivary cotinine greater than 1 ng/ml (the practical limit of quantification). Of the 8%, about two-thirds (N=27) had some other report of ETS exposure, either from post-sampling surveys (as opposed to diary reports of tobacco product smoking around them) or direct measurement of airborne nicotine. Thus, only a small fraction (2.9%) of the subjects reporting that they live and work in ETS-free environments had salivary cotinine levels above 1.0 ng/ml that cannot be reasonably ascribed to ETS exposure. If dietary sources are not resulting in higher salivary cotinine levels for Cell 4 subjects, it seems implausible that they are contributing to measurable levels in the subjects described in Table 7. This finding is also consistent with the observations reported by Benowitz (1996). Since cross-reactivity in the RIA does not appear to result in overestimation of salivary cotinine levels, and dietary contributions to systemic nicotine seem not to be important in this subject population, differences in the metabolism of nicotine in nonsmokers, compared with that of smokers, appear to be the largest contributors to the discrepancies between inhaled and systemic nicotine using the aforementioned model.

In a recent report of modeling of airborne nicotine levels to which nonsmoking workers might be exposed, and associated salivary cotinine levels, Repace et al. (1998) derived a 'rule-of-thumb' factor which could be used to estimate salivary cotinine levels from workplace-only ETS nicotine exposures. The factor was 0.05 ng/ml/μg/m^3 nicotine. This estimated conversion factor, derived from modeling area concentrations of ETS, is not confirmed by the personal monitoring data obtained in this study. For the set of subjects in this study who worked in environments where smoking was not restricted, and who had no exposure to ETS in the home (N=55), the mean average cotinine to 8-h TWA workplace nicotine concentration ratio was 1.9, and the 25th, 50th, 75th, and 90th percentile ratios were 0.11, 0.33, 1.32, and 5.77 ng/ml/μg/m^3, respectively. These are

substantially greater than the 0.05 ng/ml/$\mu g/m^3$ estimated by Repace et al. For the subset of these subjects who worked in offices only (N=29), the mean ratio was 1.9 ng/ml/$\mu g/m^3$, and the respective percentile ratios were 0.11, 0.19, 1.49, and 7.11 ng/ml/$\mu g/m^3$. The discrepancy between the finding of this study and that of the analysis by Repace et al. may be due to the latter having relied on models of area ETS concentrations to estimate personal exposure to ETS. Actual 8-h TWA levels in workplaces where smoking was unrestricted, determined via personal monitoring for subject populations in this study (Jenkins et al., 1996), are considerably lower (median nicotine=1.07 $\mu g/m^3$) than those estimated by Repace et al. (median nicotine=11.25 $\mu g/m^3$). Lower ETS nicotine levels would have the effect of increasing the value of the ratio, which is what was observed for subjects in this study.

Conclusions

Under the experimental protocols of this study, salivary cotinine is not a good quantitative indicator of airborne nicotine exposure for individual subjects. There was only minimal correlation between 24-h TWA levels of nicotine encountered by individual subjects and their individual salivary cotinine levels. Dietary contributions may play a role in elevating systemic nicotine levels, but the data from this study indicate that such is not likely at the level of sensitivity reported (mean LOD=0.55 ng/ml). Although median levels of salivary cotinine for subjects grouped according to home/work smoking status are very similar to those observed in a larger national study of serum cotinine levels (when corrected for differences between salivary and serum levels), it seems clear that the current understanding of nicotine metabolism in nonsmokers is not adequate to explain the differences between the inhaled/absorbed dose of airborne nicotine and the apparent body burden of nicotine derived from salivary cotinine levels. For larger groups of subjects, there is a strong correlation between 24-h TWA level of nicotine and salivary cotinine. This suggests that grouping subjects act together to dampen the influence of individual differences in metabolism of inhaled nicotine. To a certain extent, the grouping also acts to reduce the disparity between inhaled nicotine dose and apparent body burden. For example, using the Cell 1 data in Table 6 and assuming a daily inhalation volume of 16 m^3, the inhaled/absorbed nicotine dose, is *ca.* 23 μg/day (when dose is corrected for an estimated 71% absorption). This compares with an estimated body burden of 124 μg nicotine, based on the model described in Benowitz (1996). Thus, the difference is about a factor of 5.5, compared with a median ratio for the 263 subjects in the high cotinine/high nicotine classification of approximately 10. This may suggest that for subjects exposed to much higher levels of ETS nicotine, the comparison of inhaled/absorbed nicotine to systemic nicotine may be much closer to 1. Indeed, for the most highly exposed subjects in this study with levels of salivary cotinine indicative of indirect exposure to nicotine (18 subjects had a potential inhaled dose of nicotine—before correction for absorption—of >100 μg nicotine), the median ratio of apparent systemic nicotine to that estimated to be absorbed from ETS exposure is 2.6.

Data obtained in this study regarding the rate at which nonsmokers or never-smokers misreport their smoking status may be useful in risk assessment studies. Estimated misclassification rates for female subjects claiming to be lifetime never-smokers was 2.95%, using common discrimination criteria. This level is substantially higher than that used by the EPA in its risk assessment of lung cancer in nonsmokers, but near the median of that reported by other studies (Lee and Forey, 1995). There appear to be gender-related differences in misclassification rates, with those of males tending to be slightly higher than those of females at all discrimination levels.

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